Original Article

The effect of L-Carnitine on the recovery of achilles tendon injury in postmenopausal rats

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ABSTRACT

Background: Use of surgery for the treatment of achilles tendon rupture has its limitations. Therefore, additional treatment modalities are required during recovery of these ruptures. The present study investigated the effect of L-Carnitine (Carnitene®) on the recovery of injured achilles tendons in postmenopausal rats. **Methods:** This experimental study was conducted in 60 mature female Sprague-Dawney rats. Ovariectomy was performed for postmenopausal period. Carnitene® was administered in two doses as 100 mg/kg at low dose and 200 mg/kg at high dose using insulin injectors under sterile conditions. Inflammation score, Visual Analogue Score (VAS), and cartilaginous metaplasia were evaluated. **Results:** In rats with ovariectomy and tendon incision there was no difference in the effect of Carnitene® administration on the inflammation score (p=0.36), VAS score (p=0.26), cartilaginous metaplasia (p=0.44), and fibrosis development (p=0.44). **Conclusion:** There was no difference in the effect of the drug on postmenopausal tendon recovery. We recommend the use of Carnitene® for tendon recovery as a non-toxic and biocompatible agent.

Key words: *achilles tendon rupture, Carnitine, postmenopausal rats*

The achilles tendon recovery can be achieved using available surgical procedures to a limited extent; however, recurrent ruptures may occur during and after the recovery period. Complications of achilles tendon recovery include, prolonged return to social life, failure to achieve early movement of the affected ankle, and failure to achieve physiological recovery of the tendon. Several chemical substances, antioxidants, growth factor, medication, and agents have been used in the recovery of achilles tendons. Additional treatment modalities for the achilles tendon rupture are required, since surgical treatment still remains limited. Wound recovery declines with increasing age, which directly increases the treatment cost [1, 2].

During 1951, Frankel G et al used vitamin BT during the recovery of Achilles tendons [1]. İn the year of 1952, Cater HE et al isolated and characterized L-Carnitine [2]. In the literature, 826 studies are present which have studies the effects of L-Carnitine. The most important characteristic feature studied is that of the anti-oxidative and healing effects. L-Carnitine and its esters were evaluated that they help reduce oxidative stress and they have been proposed as a treatment for heart failure, angina and weight loss, fatigue or improving exercise performance [3]. L-Carnitine is also found to have a neuroprotective molecule and has recovery effects on the fibronectin, even if there were diabetic conditions [4, 5].

The aim of the present study was to examine one of the alternative approaches toward faster recovery and earlier return to work in repairing the Achilles tendon ruptures by using recovery effects of L-Carnitine.

MATERIAL AND METHODS

This experimental animal study was conducted after obtaining prior permission from the Ataturk University Veterinary Medicine Faculty Animal Study Department as dated 18.11.2008 with B.30.2.ATA.0.23.85-130 in ATADEM Laboratories.

This study was planned to evaluate the effects of L-Carnitine preparations (Carnitene®), on recovery of achilles tendon rupture in the postmenopausal rats. A total of 60 rats of six months old with mean weight of 200-250g were selected for the study. The rats were divided into ten groups with six rats in each group. The subjects in Group 1 were considered as the healthy control group and no procedure was performed. A total of 30 rats belonging to the groups 6, 7, 8, 9, and 10 underwent a randomized ovariectomy to reproduce experimental menopause under anesthesia. For anesthesia, 10 mg/kg ketamine hydrochloride (Ketalar®) and 5mg/mL xylazine hydrochloride (Rompun®) was administered using the intraperitoneal injection route. During both surgeries, 30 mg/kg intraperitoneal cefazolin was injected. Under anesthesia, a 2-3 cm incision was made medial to the abdominal region of the rats (Figure 1). Under sterile conditions, ovaries were bilaterally dissected and arteria ovarica and vena ovarica were tied during dissection to prevent blood loss.

Figure 1: Demonstration of Ovariectomy procedure.

Two months after ovariectomy, 36 rats (Groups 3, 4, 5, 8, 9 and 10) underwent partial dissection of the achilles tendons and were sutured under the same dose of anesthesia (Figure 2). An approximately 2 cm posteromedial longitudinal incision was made medial to the achilles tendon through the calcaneus. The incision continued through the tendon sheath, and then the tendon sheath was lifted together with the subcutaneous tissue without a subcutaneous dissection. The tendon was exposed using the mosquito-clamps and a partial incision was made. The tendon ends were sutured by a partial incision using the modified Kessler method with 5.0 non-absorbable suture material. The paratenon was sutured using 6.0 vicryl. The skin was sutured using 5.0 atraumatic silk by using a 10 amplification loop. Splint fixation was not performed.

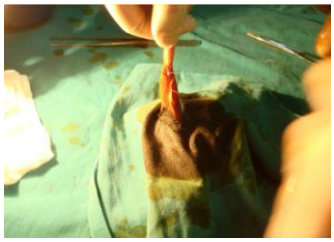

Figure 2: Achilles tendons partially dissected and sutured under the anesthesia

1g intraperitoneal Carnitene® was administered to groups 2, 4, 5, 7, 9, and 10 under sterile conditions. The rats in Groups 4, 7 and 9 were administered 100 mg/kg Carnitene® at low dose, while Groups 2, 5, and 10 received high-dose Carnitene® (200 mg/kg). The rats were fed and followed up for a month. After a month, the rats were decapitated by administering high-dose anesthesia and the amputated rats were disarticulated at the knee joint, the left Achilles tendons were surgically dissected and sutured were put into special containers containing 10% formaldehyde and their caps were, then, quickly covered and sent to the Pathology Laboratory. The samples were then fixed in paraffin-embedded blocks. Following this procedure, sections with a 5-6 micron thickness were taken using the rotator microtome and stained using hematoxylin-eosin and Masson's trichrome stains to show collagens. The preparations were assessed using an Olympus Bx50 binocular double-head light microscope.

The histopathological assesment was conducted by a single pathologist who was blind to the groups of the rats. Hematoxylin-eosin stained achilles tendon sections were assessed based on the Baransel et al's criteria on recovery [8]. The Achilles tendon recovery was examined in four categories: inflammation, vascularization, cartilaginous metaplasia, and fibrosis in dissected and re-sutured tendons. Based on the presence or absence of cartilaginous metaplasia, all findings were scored between 0 and +3 for

all other assessment criteria (Table 1).

Table 1: Histopathologic evaluation criteria on the recovery of achilles tendon

Histopathologic characteristics	Score
İnflammation (Granulocyte (PMN), Macrophage, Lymphocyte Count)	0 : inflamation cell absent +1: few +2: middle +3: lots
Vascularization	blood capillary formation
VAS score	0 : normal histological view +1: few +2: middle +3: lots
Cartilaginous metaplasia	exist absent
Fibrosis (fibrosis density)	0 : normal histological view +1: few +2: middle +3: lots

Data were analyzed using the Kruskal-Wallis test (Med Calc, Demo Version 9.6.2.0; Belgium). The main effects (non-ovariectomized vs. ovariectomized; without dissected tendon vs. with dissected tendon; 0, 100 and 200 mg/kg Carnitene® supplement) and double (ovariectomy status, tendon dissection status; ovariectomy status, Carnitene® status; tendon status, Carnitene® supplement) and triple (ovariectomy status, tendon status, Carnitene® supplement) interactions were also analyzed. The difference between the mean rank scores of the groups was considered significant at a p value of $p<0.05$.

RESULTS:

Histopathologic appearances of the groups are shown in figure 3. İn the present study, ovariectomy was not found to have any effect on the inflammation score (p=0.46), VAS score (p=0.49), cartilaginous metaplasia (p=0.99) and fibrosis development (p=0.54). Tendon incision had an effect on inflammation, VAS score, cartilaginous metaplasia, and fibrosis development (p=0.0001), all of which increased by 50% (Table 2). Carnitene® administration caused changes in all variables (p=0.04), except cartilaginous metaplasia (p=0.19).

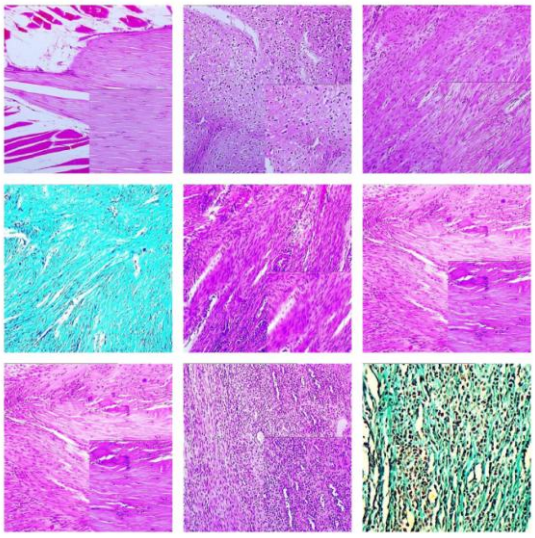

Figure 3: The light micrcroscopic view of the sections.

Ovariectomy and tendon incision influenced the inflammation score (p=0.17), VAS score (p=0.20), cartilaginous metaplasia (p=0.99), and fibrosis development (p=0.20) in a similar manner in rats with and without ovariectomy. Carnitene® administration at increasing doses in ovariectomy also influenced the inflammation score (p=0.61), VAS score (p=0.55), cartilaginous metaplasia development (p=0.74), and fibrosis development (p=0.60) in a similar manner in rats with and without ovariectomy. In those with tendon incision and Carnitene® administration at increasing doses, fibrosis development occurred at the same level in rats with and without tendon incision. In rats with tendon incision, there was a linear increase in Carnitene® dose and fibrosis score (p=0.005).

Table 2: Comparison of different groups with the histologic findings

Groups	p- value			
	Inflammation	VAS score	Cartilaginous Metaplasia	Fibrosis
Ovariectomy (O)	0.46	0.49	0.99	0.54
Tendonectomy (T)	0.0001	0.0001	0.0001	0.0001
Carnitene® (C)	0.04	0.04	0.19	0.008
OxT	0.17	0.27	0.99	0.20
OxC	0.61	0.55	0.74	0.60
TxC	0.64	0.27	0.99	0.006
OxTxC	0.36	0.27	0.44	0.44

DISCUSSION

Sex steroids are blamed for the etiology of decreased wound recovery with age [6]. Estrogen has been shown to accelerate recovery in animal models. Although the pathophysiology of the condition has been investigated, only a small part of the mechanism has been understood till date [8]. Topical and systemic estrogen treatment reduces inflammation and increases recovery rates [9]. Macrophages the primary mediators of inflammation are known to play significant roles in defense mechanism against infections, autoimmunity, tissue repair, remodelling, angiogenesis, and against cancer; they have also a key role in the production of growth factors and cytokines [9, 10].

The role of estrogen and progesterone was examined in the incisional wound recovery models induced in rats and macrophage activation was found to be responsible for wound recovery. In ovariectomy models, alternatively activated macrophage markers (FIZZ1 and Ym1) related to reduced sex steroids decreased and this effect was recovered by administering estrogen and progesterone with improved wound recovery by estrogen and progesterone. Raes G et al demonstrated that the in vivo induction of FIZZ1 and Ym1 in macrophages depends on IL-4 and that in vitro, IFN-gamma antagonizes the effect of IL-4 on the expression of FIZZ1 and Ym1 [11].

Age-related delayed wound recovery due to reduced systemic hormones in ovariectomized female mice may be associated with human wound recovery. In consistency with previous findings, Routlley et al who reported significant reduction in recovery on days 3 and 7 in ovariectomized mice due to reduced systemic hormones, compared to intact mice in full-thickness dorsal incision wounds [7]. Additionally, recovery was shown to accelerate on days 3 and 7, when exogenous estrogen and progesterone were externally administered to the ovariectomized mice. Furthermore, the total cell count prominently increased in mice with hormone displacement.

The macrophage amount within the wound was also significantly higher in these mice. Compared to ovariectomized wounds, estrogen also increased the cell count. Progesterone was not found to have an effect on the amount of macrophage around the wound. According to the authors, estrogen was clearly demonstrated to have a beneficial effect and they concluded that estrogen and progesterone, to a lesser extent, enhanced the wound recovery by increasing alternatively activated macrophage amount and breaking the local inflammatory response [8, 12].

An experimental menopause model was created in our study groups by performing ovariectomy on rats to delay the soft tissue wound recovery. Ovariectomy was performed on 30 of 60 rats included in our study. There was no histopathological effect of ovariectomy on the wound recovery in four variables: on inflammation ($p<0.46$), VAS score ($p<0.49$), cartilaginous metaplasia ($p<0.49$), and fibrosis development ($p<0.54$). Ovariectomy alone did not affect the wound recovery. Considering that the paratenon and synovia should not be greatly damaged in the rats with Achilles tendon incision models thanks to these positive effects, partial tenotomy was performed on six groups. A plaster splint was not applied after the operation.

İn our study, rats were used as they are metabolically more active, immunologically more resistant, more convenient, and easy to care for [13]. There is a common recovery mechanism in all wound recoveries including tendons. Within the tendons of tendinopathy patients, the peroxiredoxin 5 enzyme, which is known to be protective against oxidative stress, was increased and this finding supported the oxygen radicals theory [14]. Another factor involved in tendinopathy etiology is programmed cell death. Programmed cell death was demonstrated in rotator cuff tendinopathy. The specimens from ruptured supraspinatus tendons of the patients operated due to rotator cuff problems had more apoptotic fibroblast-like cells, compared to intact tendons [15].

The study by Abrahamsson with flexor tendons showed that wounds and inflammation provoked the release of growth factors and increased neovascularization, fibroblast proliferation and collagen synthesis [16]. In another study, Sarikaya observed that side effects such as tendinopathy, muscle and tendon rupture, and myopathy related to the use of atorvastatins in a rabbit model with Achilles tendon recovery [17]. Esen et al also investigated the effect of low molecular weight heparin on the rat tendon recovery and reported an increased fibroblast count, fibrillary collagen formation in the extracellular matrix, granulated endoplasmic reticulum count in the cytoplasmic content of fibroblasts with reduced mitochondrial vacuolization as a degeneration indicator [18].

For a more durable tendon after recovery, some

biomaterials were used in various studies, skeletal structures were formed from fibroblast-containing cells, and growth factors and cytokines were applied exogenously. Furthermore, tissue engineering through genes and cell therapy have been also used for this purpose [19]. Recommended options for tendinosis and peritendinitis treatment include short-term immobilization, stretching exercises, non-steroid anti-inflammatory drugs, and local heparin administration applied DMAH locally to rat Achilles tendons after crushing injuries, and reported that they histologically achieved less cohesiveness, scar formation, and had a tendon structure closer to normal [20, 21].

As an antioxidant, L-Carnitine is an important co-factor making fatty acids pass through the mitochondrial matrix in the cell. Therefore, it has an essential place in energy supply. It is a reliable drug with no known side effects. One of the studies on L-Carnitine showed that it increased the bone mineral density in the musculoskeletal system [22-24]. Chiu et al demonstrated that L-Carnitine increased the metabolic activity, thereby, increasing protein synthesis in porcine osteoblast-like cells in in vivo settings [25]. It was shown that L-Carnitine derivatives had benefits of in vivo settings, despite anti-thyroid agents in bone and similar tissues [26, 27]. As a result, the beneficial effects of L-Carnitine derivatives on the trabecular bone mass have been clearly demonstrated in rat models on a hypercalcemic diet [28]. In addition, L-Carnitine and its derivatives have been shown to conduct antioxidant and apoptotic activities in many non-osteoblast cell types [29, 30].

In our study, tendon incision significantly increased in fourvariables: inflammation, VAS scores, cartilaginous metaplasia, and fibrosis development ($p<0.0001$). Given the tendon recovery stages, this finding is consistent with the literature findings and an expected result [31]. Carnitene® administration caused significant changes in all variables, except cartilaginous metaplasia. This effect can be attributed to the metabolic effects of L-Carnitine. L-Carnitine increased fibrosis by increasing collagen synthesis. This result is consistent with the literature findings, supporting this study. It seems that directing the recovery mechanism toward intrinsic recovery, achieving recovery of scar-free-fetal quality, increasing recovery time and quality by developing molecular recovery factors, and genetic applications would gain importance in tendon injuries in the future [19]. L-Carnitine has recently been investigated in osteoporosis and wound recovery and beneficial results have been established. Limitations of our study are, usage of greater number of rats and using only 2 doses of carnitine. We recommend further studies where L-Carnitine could be applied in closer doses such as 5, 25, 50, 100 and 200, 500 mg/kg.

CONCLUSION

This experimental study found poor wound recovery in the postmenopausal period with inadequate postmenopausal Achilles tendon recovery. In addition to conservative and surgical treatments for the Achilles tendon recovery, medical treatment has become more important today. This study investigated the effect of Carnitene® on the recovery of experimental postmenopausal achilles tendon injuries, induced in rats and established histopathologically and produced statistically significant improvements. Carnitene® can be recommended in Achilles tendon ruptures at a dose of 100 mg/kg.

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